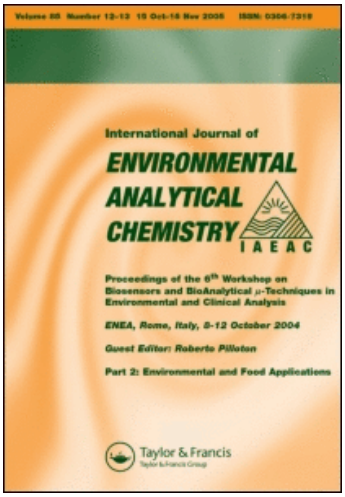

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Water-source characterization and classification with fluorescence EEM spectroscopy: PARAFAC analysis

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Water-quality protection and environmental forensics require rapid water monitoring and source identification. In this paper, parallel factor analysis (PARAFAC) of fluorescence excitation-emission matrix spectra (EEMS) was used to characterize and classify water samples from landfills, wastewater treatment plants, lakes, and rivers. The study showed that the optimal number of components was four to represent the data set. The fluorescence fingerprints for water samples from different sources were sufficiently different, so qualitative water classification could be achieved. Specifically, Component 1 was the major fluorescing centre in river waters, with characteristics consistent with humic-like fluorophores; Component 2 was the dominant fluorophore in the treated wastewaters; Component 3 was the characteristic fluorophore in landfill leachates; and Components 1, 3, and 4 existed in lake waters at comparable weight, among which Component 4 may be considered as a protein- or amino acid-like fluorophore.

Keywords: Fluorescence; Excitation and emission spectrum; EEMS; PARAFAC analysis; Water source classification

1. Introduction

During the Great Flood in 1993, 101 of the 114 counties in Missouri, US were in the Federal Disaster Declaration area. In the following year, the Solid Waste Management Program at Missouri Department of Natural Resources (MODNR) initiated a programme to investigate the impact of the flood on landfill, with an attempt to develop a rapid and effective method of identifying leachate-impacted surface water and groundwater that could be used as water supplies. Since the chemistry of landfill leachate is quite complex, but the concentrations of indicator constituents can be quite low [1], a complete analysis of the leachate constituents is often too expensive. Moreover, biodegradation, volatilization, and mixing/dilution could further complicate

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the analysis. Therefore, MODNR explored the use of fluorescence spectroscopy to trace migration of landfill leachates in the environment, since the leachate exhibited relatively strong fluorescence in the range of 250–450 nm [1]. The MODNR project showed that fluorescence spectroscopy was effective in distinguishing leachates and leachate-affected surface waters from background waters at dilution factors up to 100 and in distinguishing leachate-affected groundwater from background water in karst aquifers.

Two steps are generally involved in water-source tracking using fluorescence spectroscopy, as has been done by the MODNR project team: characterization of various waters with known sources and classification of unknown waters by comparison with the known using some characteristic parameters. A full-scan excitation-emission fluorescence spectrum of water, known as the excitation-emission matrix spectrum (EEMS), appears to contain sufficient information for water-source identification, for as much as 40–60% of natural organic matter and many contaminants themselves, such as those from the degradation of solid wastes at landfills, are source-specific fluorophores [2–5].

Fluorescence in uncontaminated river water, groundwater, and seawater, generated predominantly from natural organic matter (NOM) [2], has been applied to differentiate water sources. Two types of fluorescence are often observed: a humic acid-like fluorescence occurring at 420–450 nm from excitation at 230–260 and 320–350 nm; and a protein- or amino acid-like fluorescence with maximal emissions between 300–305 nm and 340–350 nm with excitation at 220 and 275 nm, respectively [6–9]. With this knowledge, Yan *et al*. [9] were able to successfully track river water coming from its two upstreams. Studies on fluorescence characteristics in groundwater also indicate that spatial and temporal variations in the relative wavelengths of various fluorescence centres may provide information on dissolved organic matter (DOM) source within a catchment and can be utilized as natural tracers [10]. Source-specific differences in fluorescence spectra of seawater relative to freshwater samples [7, 11], between humic substances of different origins, and between fulvic acids and humic acids from the same source [12, 13], have also been documented.

Similar to natural uncontaminated waters, numerous types of fluorescing moieties have been found in sewage wastewaters and landfill leachates. Several fluorescent constituents in sewage wastewaters have been identified, e.g. humic and lignin substances, variable amounts of steroids, phenols, non-volatile acids, oils, and trace quantities of surface-active agents [4]. The leachates from municipal landfills are also characterized by the complex chemical distribution of their constituents. Kang *et al*. [5] reported that the fluorescence spectra of humic acids extracted from landfill leachates have a relatively higher content of condensed aromatic compounds than the fulvic acids obtained from the same source.

In addition to water with well-defined sources, impacts of wastewaters on water quality can also be assessed with fluorescence spectroscopy. In a study examining the effect of discharge from sewage treatment works on the downstream river waters, Baker [3] found that the fluorescence intensities of both the fulvic-like centre (320–340 nm excitation, 410–430 nm emission) and tryptophan fluorescence centre (275 nm excitation, 350 nm emission) were significantly higher than the upstream samples for two of the investigated rivers, River Team and River Twizell Burn in England. The similarity of EEMS signatures between the downstream of River Team and River Twizell Burn and those of the outfalls demonstrated that the fluorescence signatures were preserved after mixing and dilution. In a later study involving five neighbouring rivers, including

one influenced by a tissue-mill effluent, Baker [14] found that the fluorescence spectra of the impacted water samples were dominated by tryptophan fluorescence and a fluorescence centre possibly due to fluorescent whitening agents, while the three other rivers exhibited lower fluorescence intensities typical of river systems with tryptophan (sewage), humic-like (peat derived colour), and fulvic-like (natural organic matter) sources. Fluorescence spectroscopy was also used to detect sewage pollution in a small, urbanized catchment in England, showing that over 10% of the river's discharge was provided by sewage inputs and these inputs could be grouped into clean storm water with low ammonia and tryptophan intensity, grey waters with high tryptophan intensity and low ammonia concentration, and foul waters with high tryptophan intensity and ammonia concentration [15].

The applications of fluorescence spectroscopy discussed above are mainly based on visual comparison and simple subtraction of EEMS. Such an approach, however, has significant limitations because of the constitutional complexity of many contaminated water samples and the intrinsic lack of selectivity for EEMS in general. Recent development in decomposing EEMS with several multivariate statistical methods, however, allows for more objective and expedite sample comparisons. One of these approaches is the parallel factor analysis (PARAFAC), which has been successfully used to characterize and match oil samples [16] and estuarial water samples [17–19].

In this study, PARAFAC model was applied for objective classification of waters from different sources, including lakes, landfills, wastewater-treatment plants, and rivers, based on their fluorescence EEMS. The method involved fluorescence EEMS collection, data processing, and two algorithms of PARAFAC fitting, both of which generated fingerprints based on the relative 'composition' of fluorescent components (fluorophores). As a result, we could objectively classify water samples from various sources by plotting score matrix and grouping samples with similar component scores.

2. Experimental

2.1 *Water sampling*

Four types of water from various sources, as listed in table 1, were collected and analysed for their fluorescence EEMS. Containers used for water collection (250 mL polyethylene screw-cap bottles and EPA 40 mL vials) were cleaned by soaking in 1 M HCl solution for at least 24 h and rinsed thoroughly with deionized and Milli-Q water (Millipore Inc.) prior to use. Grab samples of surface water were gathered from docks or shorelines, while leachate samples were collected from taps of landfill monitoring wells. The collected samples were refrigerated at 4°C immediately upon arrival at the laboratory for long-term use. Before performing fluorescence scans, samples were filtered through a 0.2 μm nylon syringe filter (Fisher Scientific Co.) and allowed to warm up to room temperature.

2.2 *EEM spectroscopy*

All fluorescence measurements were performed on the Hitachi F-4500 Spectrograph (Hitachi Co.). Samples were held in a standard 1-cm quartz cuvette, and the xenon lamp

Table 1. Types and locations of 15 water samples from Missouri, United States.

Landfill leachate	Jefferson City Landfill, MO (Ljc)
	North St. Louis Landfill, MO (Lnsl)
Treated wastewater (effluent)	Columbia Wastewater Treatment Plant, MO (Wcol)
	Lauerence Wastewater Treatment Plant, MO (Wlaur)
	North Topeka Wastewater Treatment Plant, MO (Wntop)
	Topeka Wastewater Treatment Plant, MO (Wtop)
Lake water	Fox Valley Lake (Kfox)
	Hazel Hill Lake (Khaz)
	Odessa Lake (Kode)
River water	Mississippi River upstream (Rmisus)
	Mississippi River downtown (Rmisms)
	Mississippi River downstream (Rmisds)
	Missouri River Jefferson City upstream (Rmizus)
	Missouri River Jefferson City midstream (Rmizms)
	Missouri River Jefferson City downstream (Rmizds)

voltage was kept constant at 700 V for all experiments. Fluorescence spectra were collected as EEMS by scanning emission spectra at a range of excitation wavelengths, in which emission spectra were gathered from 250 nm to 550 nm in 3-nm steps, whereas the excitation wavelength was stepped in 2 nm from 200 nm to 400 nm. The fluorescence scans were performed at a constant room temperature of $21 \pm 2^{\circ}$C. To eliminate the inner filtration effect on the fluorescence measurement, samples of landfill leachates and wastewater treatment plant effluents were diluted so the maximum absorbance within the whole wavelength range of EEMS scan was less than 0.05. No dilution was needed for the lake and river water samples to meet this criterion. The spectra gathered were first reported as excel files and then imported into MATLAB (version 6.5) for data analysis by using an in-house program.

2.3 *PARAFAC model*

PARAFAC model is an innovative decomposition method for EEMS based on the assumption of trilinearity of fluorescence spectra, which applies to a single excitation-emission wavelength pair with a single fluorophore and a full-scan EEMS with multi-fluorophores.

For a sample with only a single fluorophore, its fluorescence intensity can be expressed as [20]:

$$a_{ij} = 2.303\Phi_{\mathrm{f}} bcy(\lambda_i)\kappa(\lambda_i)I_0(\lambda_j)\varepsilon(\lambda_j), \tag{1}$$

where a_{ij} is the fluorescence intensity at emission wavelength λ_i and excitation wavelength λ_j; Φ_{f} the quantum efficiency of the fluorescence; b the path length of the sample; c the fluorophore concentration; $\gamma(\lambda_i)$ the fraction of fluorescence photons emitted at wavelength λ_i; $\kappa(\lambda_i)$ the wavelength dependency of the sensitivity of the analysing system, including geometrical factors, quantum efficiency of the detector, etc.; $I_0(\lambda_j)$ the intensity of exciting light incident on the sample at wavelength λ_j; and $\varepsilon(\lambda_j)$ the molar extinction coefficient of the fluorophore. An important assumption in deriving the equation (1) is that the optical density is $\ll 1$ for all λ_i.

A full-scan EEMS contains a set of $I \times J$ fluorescence intensity data, with I being the number of emission wavelengths and J the number of excitation wavelengths. If N fluorescence spectra are arranged into a three-way array, a three-way tensor A of dimensions of $N \times I \times J$ will be generated, which can be decomposed by parallel factor analysis, known as PARAFAC model. The theory and application of PARAFAC model have been well documented in the literature [18, 19, 21–24]. The decomposition of A, the three-way array of fluorescence data set with dimensions of $(N \times I \times J)$, is generally formulated as:

$$A = C(\mathrm{Em}| \otimes |\mathrm{Ex})' + E. \quad (2)$$

Here, C is the score matrix containing the concentration information of each factor (fluorophore) with dimensions of $(N \times n)$; Em the emission wavelength loading matrix with dimensions of $(I \times n)$; Ex the excitation wavelength loading matrix with dimensions of $(J \times n)$; N the number of EEMS; n the number of factors in PARAFAC model, which can literally be interpreted as the number of the types of fluorophores in all samples; and E the residual of the three-way array with dimensions of $(N \times I \times J)$.

In this study, the PARAFAC model was fitted in MATLAB using the N-way toolbox from http://www.models.kvl.dk/source. The convergence criteria and maximum number of iterations were set at 10^{-6} and 5000, respectively. The PARAFAC model was chosen because it could resolve the EEM signals of the unknown samples from that of any overlapping and uncalibrated interferents [25]. In addition, the parameters in the model, C, Em, and Ex, can be chemically interpreted as the concentration contribution from n 'pure' components (factors/fluorophores), emission spectra of the n 'pure' components, and excitation spectra of the n 'pure' components, respectively. Other multivariable models such as the N-way partial least-squares regression-discriminant analysis (NPLS-DA) have less apparent chemical meanings [17]. However, the PARAFAC model does have limitations in the permutation indeterminacy of the spectra for the n 'pure' components, the selecting of the number of factors, and the detection of outliers [17, 24]. To use PARAFAC model in this study, we removed first Rayleigh and Raman scatters and selected the proper number of components (factors). The model was then fitted with two algorithms, i.e. direct mode and calibration-test mode (http://www.models.kvl.dk/source). Source classification for the samples was finally obtained by plotting score matrix and grouping samples with similar component scores.

3. Results and discussion

3.1 *Removing Rayleigh and Raman scatters*

Rayleigh and Raman scatters cannot be modelled properly because they do not comply with the premise of trilinearity, so they need to be removed prior to PARAFAC model calculation [17, 22, 23]. The Rayleigh scatter showing up in the EEMS as straight diagonal lines is elastic, with the first-order scattered wavelengths equal to excitation wavelengths and the second-order scattered wavelengths equal to twice the excitation wavelengths. In comparison, Raman scatter is inelastic, and emission is shifted to

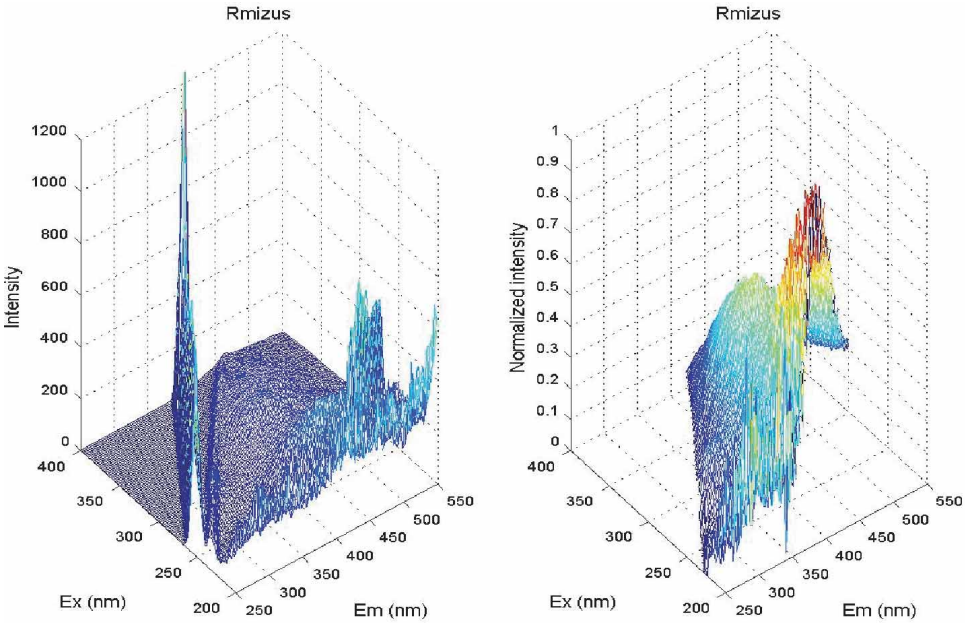

Figure 1. Fluorescence spectra of Missouri River Jefferson City upstream (Rmizus): (a) original spectrum; (b) spectrum with reduction of a blank, removal of first- and second-order Rayleigh scatters, and normalization with the highest peak remaining.

longer wavelengths compared with excitation wavelengths. Several approaches have been reported in the literature to address these scatters [16, 26, 27]. To eliminate the impact of first- and second-order Rayleigh scatters in this study, missing values (not-a-number, NaN) were inserted in the upper-right triangle of EEMS (first-order and beyond) and the lower right triangle of EEMS (second-order and beyond). In addition, the excitation-emission pairs with emission wavelengths 0–10 nm higher than excitation wavelengths of the first-order Rayleigh scatter and 0–50 nm lower than twice the excitation wavelengths of the second-order Rayleigh scatter were also replaced with NaN, which removed the data that might still be affected by Rayleigh scatters. To mitigate the impact of Raman scatter, an EEMS of blank sample (Mill-Q water) was subtracted from the EEMS of every sample. EEMS after removal of the Rayleigh and Raman scatters were normalized by dividing the spectrum by the intensity of the highest peak left. By doing this, the impact of varying DOM concentrations in different samples on the component score matrix can be reduced.

To visualize the impact of eliminating Rayleigh and Raman scatters, the original and modified fluorescence spectra of a Mississippi River sample were shown in figure 1. On the original fluorescence spectrum (figure 1a), two diagonal lines from the first-order (left) and second-order (right) Rayleigh scatters dominate the landscape. After removing these scatters, other features are visually more obvious (figure 1b).

3.2 *Determining the number of components*

For field-collected samples, determining the best number of components (factors), n, in the PARAFAC model is challenging, because it is impossible to know the exact number and nature of fluorophores present in the samples. However, if we consider that each component obtained from PARAFAC model corresponds to a defined group of fluorophores, there are several very powerful tools that can be applied to address this issue, including tools to (1) test the effect of increasing number of components on the number of iterations used to fit the model; (2) conduct the split-half analysis for two sample sets describing the same common variation; and (3) calculate the core consistency diagnostic (CORCONDIA) as a function of component number [28]. In this study, the appropriate number of components (factors) was determined to be four based on the assessments with methods (3). According to Bro [28], if the PARAFAC model is valid, the core consistency is close to 100%; if too many components are used, the core consistency will be close to zero; and if the consistency is around 50%, the model is unstable. Fitting the model with our data shows that the computed values of core consistency were 47, 75, and 92% when the number of components used was 5, 4, and 3, respectively. Further, when the selected number of components was 3, two of the three emission loadings were bimodal, indicating that the number of components chosen was too small. When the number of components was chosen to be 4, only one of the four emission loadings was bimodal (as shown later in figure 2), suggesting that four components could represent the data set most appropriately.

3.3 *Fitting the model with direct mode*

To qualitatively characterize and classify the samples, we stacked all 15 samples examined into a three-way tensor and fitted PARAFAC model with the data. The excitation loading and emission loading of the four components are illustrated in figure 2(a).

It appears that some identified fluorescing components in this study could be assigned to the fluorescing centres that have been previously identified in natural waters and anthropogenic waters. Component 1 has an emission fluorescent centre at 450–480 nm from excitation at 250 nm (figure 2a), which is consistent with the features of UV fluorescing humic-like spectra as reported by Hall *et al*. [17]. There is also a shoulder at about 330–360 nm, which could be considered as visible fluorescing humic acids [29]. This component exhibits emission at a longer wavelength than other components, suggesting that it is composed of the DOM with more conjugated molecules [30]. Component 2 has the most complicated excitation and emission spectra. Two excitation peaks with maxima at 275 and 350 nm, as well as an excitation peak that is below the 200 nm, the lower limit of our EEM scan, were observed. The emission spectrum comprises two fluorescent peaks centred at 300 and 450 nm, respectively. Notice that Component 2 contains an emission peak and an excitation peak at shorter wavelengths than those in other components, suggesting that the DOM in Component 2 is less conjugated or contains few functional groups [18, 29, 30]. The multiple-band characteristics of the spectra indicate the presence of greatly differing fluorophores in Component 2 [18, 19]. Since a relatively small number of samples from quite distinct

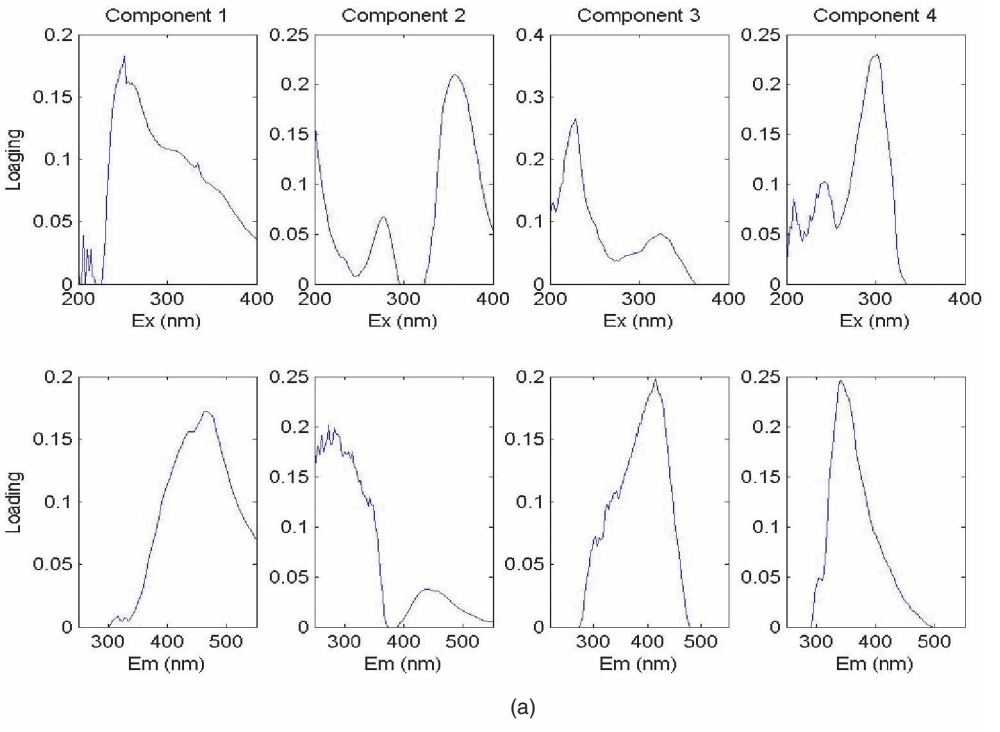

Figure 2. Excitation and emission loadings of the samples: (a) direct mode; (b) calibration-test mode.

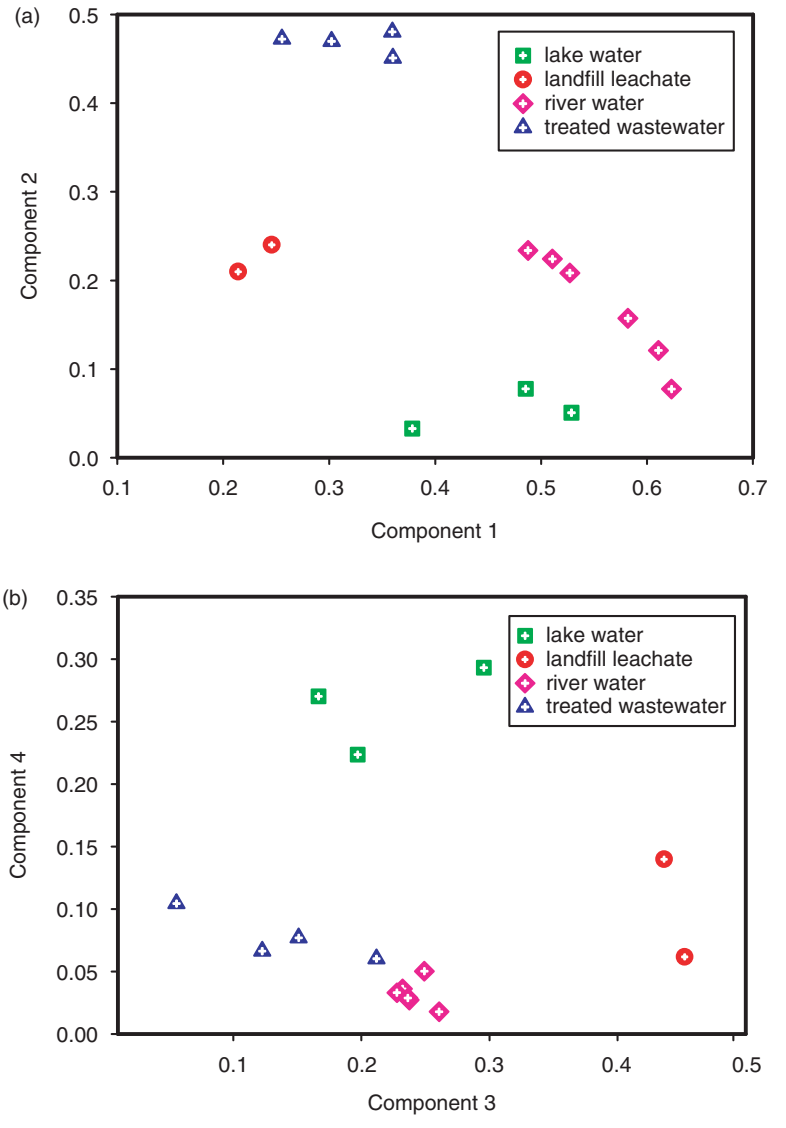

Figure 3. Water source identification by grouping component scores (direct mode): (a) source classification: Component 1 *vs*. Component 2; (b) source classification: Component 3 *vs*. Component 4.

location and sources were used, this component could not be decomposed into two or even more different peaks. Component 3 is characterized with double excitation maxima at 230 and 325 nm, and a single, well-defined emission peak at 410–430 nm. Component 4 may be considered as a protein- or amino acid-like fluorophore with a fluorescent centre at 330–350 nm emission from excitation at 240 and 300 nm [6–9]. In short, different components generally show substantially different excitation and emission loadings, but some overlaps exist such as between the emission loadings of Components 3 and 4.

The score matrix of these samples is shown in figure 3 as two plots: (a) Component 1 *versus* Component 2, and (b) Component 3 *versus* Component 4. Notice that the score

matrix could also be plotted in other combinations, e.g. Component 1 *versus* Component 3 and Component 2 *versus* Component 4. It is clear that different fluorophores dominate in different waters, which therefore provides a basis for the water source classification. In the river waters, Component 1 is the dominant fluorophore. A similar fluorophore has been found in samples from forested and wetland regions [18]. In the treated wastewater, Component 2 is the dominant fluorophore. As discussed above, the spectra of Component 2 indicate the presence of DOM with less conjugated/less aromatic or with fewer functional groups than those in the other components [18, 29, 30]. This suggests that most of the DOM in the treated wastewater is probably made of low-molecular-weight organic compounds that have not gone through the repeated decomposition/polymerization processes responsible for humic acid formation. In the landfill leachate, Component 3 dominates, which has often been identified in terrestrially dominated end-member samples, originating from terrestrially derived organic matter [9, 18, 19], but also observed in some marine water samples, originating from marine humic matter [6–8]. In the lake waters, Components 1, 3, and 4 are present at almost the same weight, which may reflect complex sources of these lakes.

3.4 *Fitting the model with calibration-test mode*

Source identification of unknown water samples can also be performed with the other algorithm, called the calibration-test mode. This involves fitting the model with data from samples with known sources and calculating the score matrix, and then classifying the unknown samples by plotting the score matrix and grouping samples with similar component scores. In a sense, this algorithm is closer to identification of unknown samples with a database of known sources. As a demonstration, we arbitrarily chose one lake water (Kode), one treated wastewater (Wtop), and two river-water samples (Rmisus and Rmizus) as having unknown sources. Fitting the PARAFAC model with the remaining 11 samples in the same way as we did using the direct mode, we obtained excitation loading and emission loading, as shown in figure 2(b). Of note is that the excitation and emission loadings calculated with these two modes are almost identical (figure 2a and figure 2b), which can be taken as a strong validation for the application of the model. The computed score matrix is illustrated in figure 4(a) and (b). This process can be referred to as the calibration process, which provides 'standard' excitation and emission loadings. By inserting the computed excitation loading and emission loading into the PARAFAC model, the score matrix for the four unknown samples was calculated and illustrated in figure 4(c) and (d). In the process of this test, all unknown samples can be easily classified by comparison with the corresponding source categories.

4. Conclusions

In summary, EEMS provide rich information that allows rapid qualitative classification of water sources through two algorithms of PARAFAC modelling. For the 15 waters

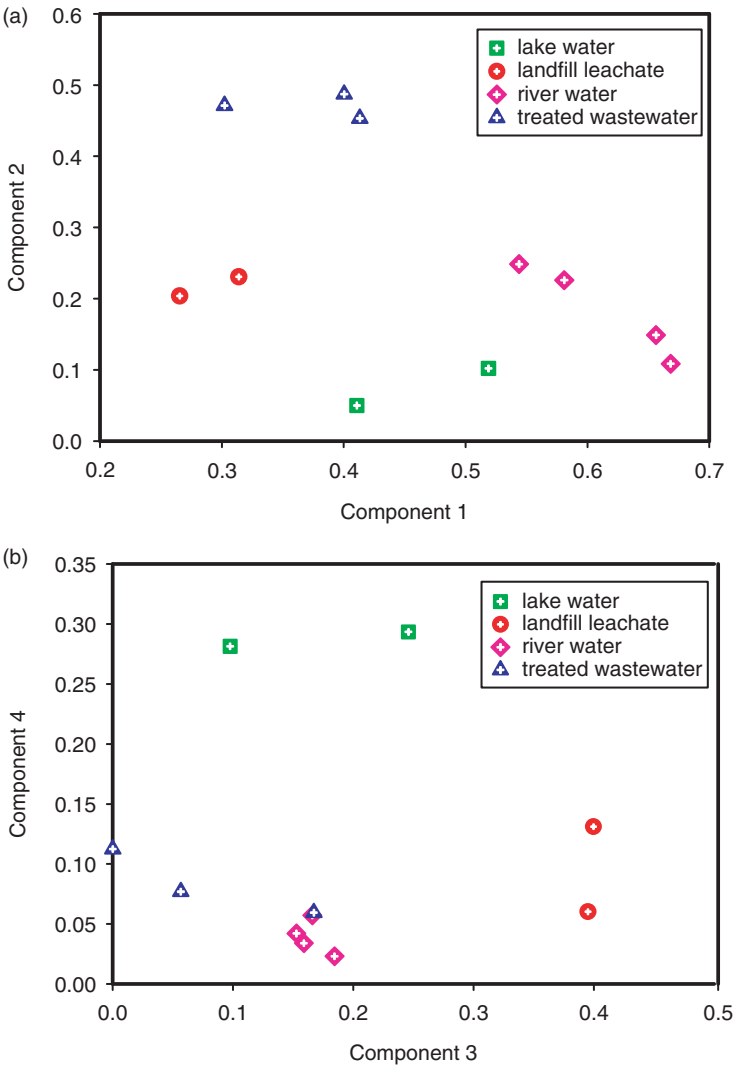

Figure 4. Water-source identification by grouping component scores (calibration-test mode). (a) Source classification: Comp. 1 *vs*. Comp. 2 (calibration). (b) Source classification: Comp. 3 *vs*. Comp. 4 (calibration). (c) Source classification: Comp. 1 *vs*. Comp. 2 (test). (d) Source classification: Comp. 3 *vs*. Comp. 4 (test).

examined, four components are optimal to represent the data set. With different components dominating in different waters, the water source characterization and classification could be readily carried out. It is clear that the established procedure is objective and does not rely on the experience of individual analysts for visual sample comparisons, as has often been practised. The results from the PARAFAC model provide information on the concentration, emission wavelength, and excitation wavelength of each component. Since the fluorescence EEM technique is much more

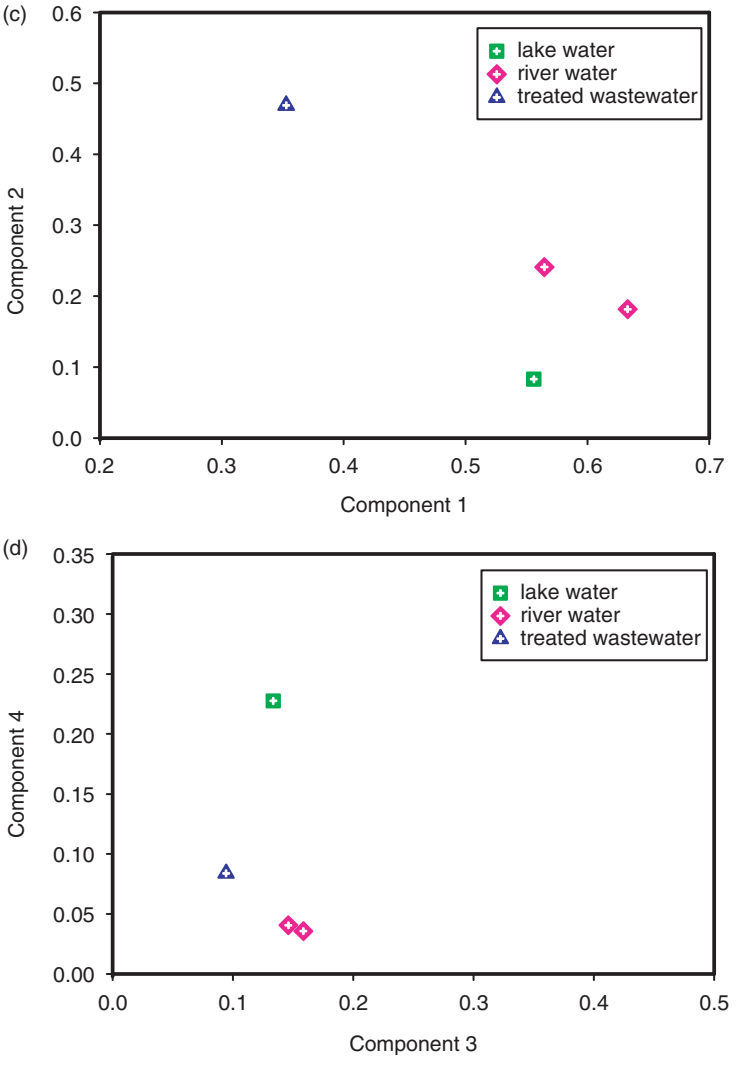

Figure 4. Continued.

sensitive than absorbance measurement, and the modern fluorescence spectrometer allows rapid EEMS data acquisition, it is potentially possible for online monitoring of wastewater treatment processes.

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